

## Genetic Linkage of Hyper-IgE Syndrome to Chromosome 4

Bodo Grimbacher,<sup>1</sup> Alejandro A. Schäffer,<sup>3</sup> Steven M. Holland,<sup>4</sup> Joie Davis,<sup>2</sup> John I. Gallin,<sup>4</sup> Harry L. Malech,<sup>4</sup> T. Prescott Atkinson,<sup>5</sup> Bernd H. Belohradsky,<sup>13</sup> Rebecca H. Buckley,<sup>6</sup> Fausto Cossu,<sup>11</sup> Teresa Español,<sup>9</sup> Ben-Zion Garty,<sup>12</sup> Nuria Matamoros,<sup>10</sup> Laurie A. Myers,<sup>6</sup> Robert P. Nelson,<sup>7</sup> Hans D. Ochs,<sup>8</sup> Eleonore D. Renner,<sup>13</sup> Nele Wellinghausen,<sup>14</sup> and Jennifer M. Puck<sup>1</sup>

<sup>1</sup>Genetics and Molecular Biology Branch and <sup>2</sup>Medical Genetics Branch, National Human Genome Research Institute, <sup>3</sup>Computational Biology Branch, National Center for Biotechnology Information, and <sup>4</sup>Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; <sup>5</sup>Department of Pediatrics, University of Alabama at Birmingham, Birmingham; <sup>6</sup>Department of Pediatrics and Immunology, Duke University Medical Center, Durham, North Carolina; <sup>7</sup>Division of Allergy and Clinical Immunology, University of South Florida/All Children's Hospital, St. Petersburg; <sup>8</sup>Department of Pediatrics, University of Washington, Seattle; <sup>9</sup>Immunology Unit, Hospital Vall d'Hebron, Barcelona, and <sup>10</sup>Department of Immunology, Hospital Son Dureta, Palma de Mallorca, Spain; <sup>11</sup>Bone Marrow Transplant Unit, Ospedale Microcitico, Cagliari, Italy; <sup>12</sup>Schneider's Children Medical Center of Israel, Sackler Faculty of Medicine, University of Tel Aviv, Tel Aviv; and <sup>13</sup>Section of Infectious Diseases and Clinical Immunology, Dr. von Haunersches Kinderspital, Munich, and <sup>14</sup>Section of Infectious Diseases and Clinical Immunology, University of Ulm, Ulm, Germany

### Summary

The hyper-IgE syndrome (HIES) is a rare primary immunodeficiency characterized by recurrent skin abscesses, pneumonia, and highly elevated levels of serum IgE. HIES is now recognized as a multisystem disorder, with nonimmunologic abnormalities of the dentition, bones, and connective tissue. HIES can be transmitted as an autosomal dominant trait with variable expressivity. Nineteen kindreds with multiple cases of HIES were scored for clinical and laboratory findings and were genotyped with polymorphic markers in a candidate region on human chromosome 4. Linkage analysis showed a maximum two-point LOD score of 3.61 at recombination fraction of 0 with marker D4S428. Multipoint analysis and simulation testing confirmed that the proximal 4q region contains a disease locus for HIES.

### Introduction

Hyper-IgE syndrome (HIES), also called “Job syndrome” (MIM 147060) and “hyper-IgE recurrent infection syndrome” (243700), was first described as a primary immunodeficiency characterized by recurrent

staphylococcal skin abscesses, recurrent pneumonia with pneumatocele formation, eczema, eosinophilia, and highly elevated levels of serum IgE (Davis et al. 1966; Buckley et al. 1972; Hill et al. 1974a, 1974b; Donabedian and Gallin 1983; Belohradsky et al. 1987). A distinctive facial appearance, hyperextensibility of the joints, bone fractures, and craniosynostosis have been reported in many cases of HIES (Davis et al. 1966; Kirchner et al. 1985; Cohen 1988; Borges et al. 1998; Holland and Gallin 1998). Recently, a systematic evaluation of 30 patients established HIES as a multisystem disorder characterized by susceptibility to infection, elevated levels of serum IgE, eosinophilia, a unique facial phenotype, retained primary dentition, bone fragility, hyperextensible joints, and scoliosis (Grimbacher et al. 1999a).

Most patients with HIES are sporadic cases. However, in many kindreds, autosomal dominant transmission, including male to male transmission, has been reported (Van Scoy et al. 1975; Blum et al. 1977; Buckley and Sampson 1981; Kraemer et al. 1981; Dreskin and Gallin 1987; Buckley 1996; Grimbacher et al. 1999a). In addition, the expressivity of HIES within a kindred can be highly variable.

Past studies of HIES focused on the immune system. Investigations targeted IgE production and metabolism (Buckley and Becker 1978; Buckley et al. 1982; Kraemer et al. 1982; Ochs et al. 1983; Dreskin et al. 1985, 1987; Vercelli et al. 1990); granulocyte chemotaxis (Hill et al. 1974a, 1974b; Van Scoy et al. 1975; Van Epps et al. 1983; Gahr et al. 1987); eosinophilia (Buckley and Sampson 1981; Donabedian and Gallin 1983; Buckley 1996); T-lymphocyte subsets (Geha et al. 1981; Ochs et al. 1983; Buckley et al. 1991); production of cytokines, such as interleukin 4 and interferon  $\gamma$  (King et al. 1989; Claassen et al. 1991; Paganelli et al. 1991; Rousset et

Received May 25, 1999; accepted for publication July 14, 1999; electronically published August 11, 1999.

Address for correspondence and reprints: Dr. Jennifer M. Puck, Chief, Immunologic Genetics Section, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, Building 49, Room 3A14, 49 Convent Drive, Bethesda, MD 20892-4442. E-mail: [jpuck@nhgri.nih.gov](mailto:jpuck@nhgri.nih.gov)

© 1999 by The American Society of Human Genetics. All rights reserved.  
0002-9297/1999/6503-0019\$02.00

al. 1991); and responsiveness to cytokine-mediated signals (e.g., via the interleukin-4 or interferon- $\gamma$  receptors) (Hershey et al. 1997). However, no immunologic defect has been found consistently in all patients. Moreover, candidate-gene approaches, to date, have failed to demonstrate linkage of HIES to the locus of the interleukin-4 receptor on chromosome 16 (Grimbacher et al. 1998a).

Thus, the primary cause of HIES remains unknown. Our demonstration of the involvement of many systems in HIES suggests that the gene(s) responsible for HIES must be involved not only in the regulation of IgE and inflammation but also in the development and remodeling of bone and soft tissue.

To pursue the genetic localization of HIES, affected individuals and their relatives were evaluated, a quantitative-phenotype score was developed, and cytogenetic analyses were performed. One patient with sporadic HIES plus autism and mental retardation was found to have a supernumerary marker chromosome, derived from a 15-20-cM interstitial deletion in chromosome 4q21 (Grimbacher et al. 1999b). This information prompted an investigation of linkage of HIES with polymorphic markers on chromosome 4. Here, we report linkage of our multiplex HIES families to the cytogenetically identified candidate region on chromosome 4.

## Subjects and Methods

### *Subjects and Families*

Forty-four fully affected subjects with HIES and 93 of their relatives, eight of whom were considered to have a mild form of HIES, were evaluated by physicians of the National Institutes of Health (NIH; B.G., S.M.H., and J.M.P.) in compliance with approved protocols (95-HG-0066 and 93-I-0119). This cohort was used to develop a phenotypic questionnaire and scoring system. Twenty-four patients with HIES were from 12 unrelated multiplex white, Asian, and African American families (see fig. 1; families 1-7, 9, 15, 17, 20, and 22). The remaining 20 patients with HIES were sporadic cases. In addition, multiplex HIES kindreds were sought from collaborating immunologists, and an additional seven HIES families were enrolled (see fig. 1; families 10-12, 18, 19, 21, and 24). Families 8, 13-15, and 23 were not used for linkage analysis, either because there was only one definite case of HIES or because the second case was an identical twin of the proband. Informed consent was obtained from all subjects or their parents. Histories were recorded; physical examinations, complete and differential blood counts, and IgE determinations were performed; and blood samples for karyotype and DNA analyses were obtained. DNA was extracted from whole

blood with the Puregene DNA Isolation Kit (Gentra Systems).

### *Scoring System*

To account for the phenotypic variability in relatives of probands with HIES, a scoring system was developed with use of both clinical and laboratory test criteria (table 1). Five of us (J.I.G., B.G., S.M.H., H.L.M., and J.M.P.) used the literature and recent analysis of 30 patients with HIES and 70 of their relatives (Grimbacher et al. 1999a) to assign a point value for each finding, on the basis of its incidence in HIES and specificity for HIES. Findings highly specific to HIES were given more weight than those that are frequent in HIES but that also are relatively common in the general population. Individual clinical impressions of the above physicians were consistent with the following total-point score assignments: at  $\geq 15$  points, the subject is likely to carry an HIES genotype; at 10-14 points, the presence of an HIES genotype is indeterminate; and at  $< 10$  points, the subject is unlikely to have an HIES genotype. Some of the clinical findings, such as scoliosis, the characteristic facies, and retained primary teeth, cannot be ascertained in children with age  $< 8$  years and may first appear during adolescence. Similarly, the number of episodes of infections and fractures and the chance of developing pneumatocoles in patients with HIES increases as patients get older. To reflect this diagnostic uncertainty and to avoid false-negative scores in children, inversely age-dependent points were assigned to individuals with age  $< 5$  years. This scoring system was validated in 14 additional newly enrolled NIH patients plus their family members and in 16 patients with HIES and their relatives who were evaluated at the Dr. von Haunersches Kinderspital (by B.H.B. and E.D.R.) (Belohradsky et al. 1987). Scores within each family were assigned by NIH clinical investigators before genotypes for that family were analyzed.

### *Genotyping*

Fluorescent primers for polymorphic markers from chromosome 4 were purchased from Research Genetics and PE Applied Biosystems. Genotyping PCR was performed at the conditions specified for each primer set. PCR results were analyzed on an ABI 377 sequencer (PE Applied Biosystems) with the COLLECTION and ANALYSIS software packages (PE Applied Biosystems). Allele sizes were determined with the help of GENOTYPER (PE Applied Biosystems).

### *Linkage Analysis*

For linkage analysis, individuals were assigned to different liability classes on the basis of their clinical score



**Table 1**

**Scoring System with Clinical and Laboratory Tests for Individuals in Kindreds with HIES**

CLINICAL FINDINGS	POINTS <sup>a</sup>									
	0	1	2	3	4	5	6	7	8	10
Highest serum-IgE level (IU/ml) <sup>b</sup>	<200	200–500			501–1,000				1,001–2,000	>2,000
Skin abscesses	None		1–2		3–4				>4	
Pneumonia (episodes over lifetime)	None		1		2		3		>3	
Parenchymal lung anomalies	Absent						Bronchiectasis		Pneumatocele	
Retained primary teeth	None	1	2		3				>3	
Scoliosis, maximum curvature	<10°		10–14°		15°–20°				>20°	
Fractures with minor trauma	None				1–2				>2	
Highest eosinophil count (cells/ $\mu$ l) <sup>c</sup>	<700			700–800			>800			
Characteristic face	Absent		Mildly present			Present				
Midline anomaly <sup>d</sup>	Absent					Present				
Newborn rash	Absent				Present					
Eczema (worst stage)	Absent	Mild	Moderate		Severe					
Upper respiratory infections per year	1–2	3	4–6		>6					
Candidiasis	None	Oral	Fingernails		Systemic					
Other serious infections	None				Severe					
Fatal infection	Absent				Present					
Hyperextensibility	Absent				Present					
Lymphoma	Absent				Present					
Increased nasal width <sup>e</sup>	<1 SD	1–2 SD		>2 SD						
High palate	Absent		Present							
Young-age correction	>5 years			2–5 years		1–2 years		≤1 year		

<sup>a</sup> The entry in the furthest-right column is assigned the maximum points allowed for each finding.

<sup>b</sup> Normal <130 IU/ml.

<sup>c</sup> 700/ $\mu$ l = 1SD, 800/ $\mu$ l = 2 SD above the mean value for normal individuals.

<sup>d</sup> For example, cleft palate, cleft tongue, hemivertebrae, other vertebral anomaly, etc. (see Grimbacher et al. 1999a).

<sup>e</sup> Compared with age- and sex-matched controls (see Farkas et al. 1994).

(table 2). Linkage calculations were done with FASTLINK version 4.0P (Cottingham et al. 1993; Schäffer et al. 1994), a faster version of LINKAGE 5.1 (Lathrop et al. 1984), and with FASTSLINK (Ott 1989; Weeks et al. 1990; Cottingham et al. 1993). Most of the analyses were done in individuals assigned to penetrance classes as shown in table 2. We assumed that all founders who married into families in which the disease was already present could not be carrying the disease allele. The penetrance values in table 2 were assigned on the basis of the method advocated by Terwilliger and Ott (1994) for a disease in which there is significant diagnostic uncertainty. For example, in the affected class with penetrance values of 0.1, 0.9, and 0.9, we were 90% certain of a diagnosis of an HIES genotype. In some analyses, we applied alternative boundaries between the two classes with the highest clinical score. The disease-allele frequency was set at  $1 \times 10^{-6}$ , on the basis of the empirical estimation that the number of fully affected cases in the United States and Europe appears to be in the low 100s. Allele frequencies were estimated with the ILINK program of FASTLINK. Two-point analyses were done with the MLINK and ILINK programs of FASTLINK. Multipoint analyses were done with the LINKMAP program, on the basis of intermarker recombination fractions ( $\theta$ ) estimated by ILINK. Locus-heterogeneity tests were done with the MULTIHOMOG program of the ANALYZE package (Terwilliger 1995).

#### Maps and Marker Loci

We determined the order of the polymorphic markers by comparing maps and marker loci from the following databases: Genemap'98, Whitehead Institute for Biomedical Research-MIT Center for Genome Research, and Research Genetics Inc. (Stewart et al. 1997). ILINK was used to establish the likely marker order in our data set.

## Results

#### Application of the Scoring System

The clinical and laboratory scoring system (table 1) was applied to all available members of the 19 families enrolled in this study. Although the theoretical maximum HIES score is 109, the most-severely affected proband achieved a score of 79 (fig. 1, family 5). Of 91 individuals at risk to inherit an HIES genotype (see fig. 1), 46 had scores of  $>14$  points and were given a classification of "affected," with various penetrance classes (table 2). Seven subjects had scores of 10–14 and were given a classification of "unknown." Thirty-eight had scores of  $<10$  and were given a classification of "unaffected." These numbers, although derived solely from clinical and laboratory data for each individual, reflect

**Table 2**

**Liability-Class Assignment on the Basis of HIES Phenotypic Score**

CLINICAL POINT SCORE	PHENOTYPE	PENETRANCE <sup>a</sup>		
		w/w	w/m	m/m
$\geq 61$	Affected	.00	.999	.999
51–60	Affected	.01	.99	.99
41–50	Affected	.02	.98	.98
31–40	Affected	.03	.97	.97
21–30	Affected	.05	.95	.95
18–20	Affected	.10	.90	.90
15–17	Affected	.20	.80	.80
10–14	Unknown	...	...	...
8–9	Unaffected	.80	.20	.20
6–7	Unaffected	.90	.10	.10
4–5	Unaffected	.98	.02	.02
0–3	Unaffected	.99	.01	.01
...	Married in, unaffected	.999	.00	.00
...	Unavailable, unknown	...	...	...

<sup>a</sup> Fractions represent likelihood of autosomal dominant HIES genotypes. Abbreviations: "w/w" = homozygous wild type, "w/m" = heterozygous wild type/mutant, and "m/m" = homozygous mutant. (In conventional LINKAGE notation, the three probabilities for scores of 0–9 would be in the reverse order.)

the expected segregation of an autosomal dominant trait.

#### Linkage Analysis

Table 3 shows the two-point LOD scores for each of 18 polymorphic markers on chromosome 4, compared with a dominant HIES phenotype as defined in table 2. The maximum LOD score of 3.61 was achieved with marker D4S428 at  $\theta = 0$ . Approximately 0.4 of this total LOD score was a result of fortuitous genotypes in families 3 and 15 at marker D4S428; these two families have lower scores at all nearby markers. Therefore, we expected multipoint LOD scores with marker D4S428 to be somewhat lower than 3.6. Table 4 shows representative maximum point-point LOD scores of  $\sim 3.4$ . These multipoint calculations were done with two of the more informative markers (D4S1547 and D4S1627) on the proximal side of D4S428 and with three of the more informative markers (D4S3248, D4S2638, and D4S398) on the distal side. The maximum multipoint LOD score was near 3.4 regardless of which flanking markers were used. Figure 2 shows a graph of representative multipoint LOD scores, in this case with more-widely spaced markers D4S405, D4S428, and D4S1534. As expected, an analysis in affecteds only gave positive LOD scores in this region, but with values  $<1.5$ , indicating a lack of power.

The two-point results from our data set were sensitive to the choice of the penetrances because each family was small, there were few affected individuals, and the di-

Table 3

## Two-Point Linkage Analysis of HIES with Markers on Chromosome 4

MARKER	DISTANCE FROM 4PTCL (IN CM) <sup>a</sup>	LOD SCORE AT $\theta =$					MAXIMUM LOD SCORE	$\theta_{\max}$
		.00	.01	.05	.10	.20		
D4S405	56	1.88	1.88	1.84	1.70	1.26	1.88	.012
D4S2919	57	.86	.89	.94	.92	.73	.94	.065
D4S1547	60	2.13	2.08	1.91	1.68	1.22	2.13	.000
D4S1627	60	2.65	2.62	2.48	2.25	1.67	2.65	.000
D4S401	60	.41	.42	.41	.39	.31	.42	.021
D4S1577	61	1.26	1.27	1.28	1.23	1.02	1.28	.034
D4S2996	62	1.06	1.07	1.08	1.04	.87	1.08	.039
D4S1630	63	2.25	2.20	2.03	1.81	1.32	2.25	.000
D4S428	63	3.61	3.54	3.26	2.89	2.10	3.61	.000
D4S3000	67	.94	1.05	1.29	1.36	1.20	1.36	.102
D4S1592	68	.91	1.01	1.18	1.19	.94	1.21	.075
D4S3248	72	1.28	1.35	1.44	1.39	1.08	1.44	.055
D4S2638	72	1.54	1.63	1.76	1.73	1.40	1.77	.064
D4S398	72	2.53	2.48	2.27	2.00	1.43	2.53	.000
D4S2367	77	.64	.75	.96	1.02	.86	1.02	.095
D4S392	78	.98	1.07	1.24	1.26	1.06	1.27	.084
D4S1534	95	-2.13	-1.23	-.29	.16	.46	.47	.234
D4S423	100	-1.26	-.95	-.42	-.15	.06	.09	.276

<sup>a</sup> Distances are from Broman et al. (1998).

agnosis was usually not very certain in all family members. Indeed, roughly half the families showed no LOD score outside the range ( $-0.01$ – $+0.01$ ), because the phenotype score dictated assignment to penetrance classes with considerable diagnostic uncertainty. Table 5 shows how the maximum two-point LOD score with marker D4S428 changed as we varied the lowest affected phenotype score assigned to penetrance values of 0.00, 0.999, and 0.999. Moreover, table 5 suggests that several of the families with peak clinical scores  $<60$  may not be linked to chromosome 4, although formal tests of locus heterogeneity were inconclusive.

Because the maximum LOD score was not much above the standard threshold of 3.0, and because the maximum LOD score was sensitive to the choice of the penetrance, we also used simulation to test the significance of the scores generated by our data. There were 10 families in our collection (families 1–5, 10, 15, 18, 19, and 22) that exhibited a two-point LOD score with

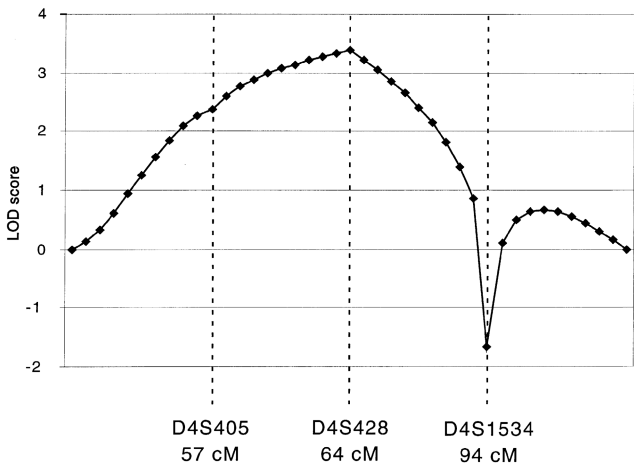
an absolute value of  $\geq 0.01$ . We used FASTLINK to generate marker data for 7,000 replicates of these 10 families in which phenotypes and penetrances were as in table 2, and genotypes were assigned at random for one unlinked marker having exactly the allele frequencies we estimated for marker D4S428. For each of the 7,000 replicates, we computed the two-point LOD score at  $\theta = 0.0$ – $0.1$ , in increments of 0.01. Then we asked, how often did the best of these LOD scores for a replicate exceed various thresholds? The results of this test are shown in table 6. The maximum LOD score for the unlinked replicates never reached the observed multipoint LOD score of 3.46 or the observed two-point LOD score of 3.61. In fact, the highest LOD score obtained, by any unlinked replicate, was  $< 3.1$ . To assess the significance of seeing zero unlinked replicates in 7,000 with LOD scores  $> 3.46$ , we used the method of Ott (1991). Let  $X$  be a binomial random variable with probability  $p$  of success, such that  $X$  is the event of having a LOD score  $> 3.46$  in an unlinked replicate. In this representation, we observed zero successes in 7,000 binomial trials. The upper boundary of a 95% confidence interval for the estimate of  $p$  is  $< .0005$ .

Linkage calculations in our family data were also performed with a model of recessive inheritance, with a carrier frequency of .001 corresponding to the assumed incidence of HIES of  $1/10^6$ . The LOD scores for most of the 18 markers were negative, but of a magnitude sufficiently small to exclude only the hypothesis that all families are linked to 4q with recessive inheritance. No

Table 4

## Four-Point Linkage Analysis of HIES to Markers on Chromosome 4

Markers Used	Maximum Four-Point LOD Score
D4S1547-D4S428-D4S3248	3.46
D4S1547-D4S428-D4S2638	3.39
D4S1547-D4S428-D4S398	3.39
D4S1627-D4S428-D4S3248	3.41
D4S1627-D4S428-D4S2638	3.41
D4S1627-D4S428-D4S398	3.40



**Figure 2** Four-point linkage analysis of HIES. LOD scores were calculated at the points shown, and linear interpolation was used in between.

conclusions could be made about the likelihood of recessive inheritance in individual families.

**Discussion**

Analysis of known immunologic pathways has failed to elucidate the nature of the underlying defect in HIES. Candidate-gene approaches, to date, have been unsuccessful (Hershey et al. 1997; Grimbacher et al. 1998), and positional cloning was not previously feasible because of the lack of multiplex kindreds with this primary immunodeficiency, due to early fatality in the era before effective antibiotic treatment. We have collected 19 multiplex HIES kindreds, enabling us not only to study the phenotype and genetics of the disease (Grimbacher et al. 1999a) but also to conduct genetic-linkage studies as presented here.

The observation of a cytogenetic anomaly, an interstitial deletion and marker-chromosome formation in chromosome 4q, in a sporadic HIES patient with mental retardation and autism (Grimbacher et al. 1999b) prompted us to conduct a limited linkage study in this region of chromosome 4. Here, we report linkage of our HIES families to chromosome 4 with a maximum multipoint LOD score >3.4, with significance confirmed by simulation.

To assign the individuals enrolled in the present linkage study to penetrance classes, we developed a scoring system, on the basis of fully affected patients and their relatives, using clinical data and laboratory tests. This system assigns the most points to findings specific to HIES, such as failure of dental exfoliation, serum-IgE levels >10,000 IU/ml, or pneumatocoe formation. Findings of HIES that are also common in the general pop-

ulation, such as recurrent upper respiratory infections, serum-IgE levels <500–1,000 IU/ml, or eczema, received fewer points. This scoring system was validated in newly enrolled NIH patients and in an independent HIES cohort in Germany.

Using this model, we were able to demonstrate linkage of HIES in our families to the candidate region on chromosome 4. At most of the loci, seven of the families (families 1–5, 10, and 19) contributed positive two-point LOD scores > 0.01, and three of the families (families 15, 18, and 22) contributed negative two-point LOD scores <–0.01, giving a hint of locus heterogeneity. For small pedigrees, such as those reported here, and for small recombination fractions, the absolute value of negative scores in families inconsistent with tight linkage tends to be much larger than the absolute value of the positive scores in families consistent with linkage. This explains why the LOD scores decreased as the criteria for diagnostic certainty were relaxed (table 5). As the threshold for certain diagnosis was lowered, from 63 to 48 points, more families contributed notable positive and negative scores, but the negative scores were much larger in absolute value at small recombination fractions. In such a circumstance, and with a maximum LOD score < 4, it is mathematically impossible to establish locus heterogeneity by the inference procedure of HOMOG/MULTIHOMOG (Terwilliger and Ott 1994; Terwilliger 1995). This is because the inference procedure dampens the effect of the small positive scores by a larger fraction than does the effect of the larger (in absolute value) negative scores.

Many families did not contribute LOD scores of an absolute value ≥0.01 because of the combination of few cases in the family, diagnostic uncertainty, and low disease-allele frequency. Nonetheless, our multipoint LOD scores >3.4 exceeded the standard genomewide significance threshold of 3.0. One could argue for a much

**Table 5**  
Variation in Maximum Two-Point LOD Score for Marker D4S428 Depending on Boundary between the Two Most Extreme Affected Liability Classes

Lowest Phenotype Score Assigned to Penetrance Values of .00, .999, and .999	Maximum LOD Score	$\theta$
63	3.37	.0
59–62	3.61	.0
56–58	3.61	.0
54–55	2.79	.05
53	2.32	.08
50–52	2.41	.08
49	2.01	.128
48	1.93	.144

**Table 6**

**Number of Unlinked Replicates of 7,000 Giving a Maximum Two-Point LOD Score Exceeding Various Thresholds**

Threshold	No. Exceeding Threshold
3.1	0
3.0	1
2.5	3
2.0	7
1.5	34
1.0	130
.5	373
.0	897

smaller LOD-score threshold in this circumstance, in which only a small candidate interval was tested. Moreover, we confirmed the significance of the two-point scores by a simulation in which none of 7,000 unlinked replicates reached a two-point LOD score  $\geq 3.1$ .

Since we were led to suspect chromosome 4q as a locus for HIES because of a cytogenetic abnormality in one patient with sporadic HIES, it is necessary to ask how the linkage region compares with the interval involved in the interstitial deletion and marker-chromosome formation in 4q21. Such analysis is complicated by the fact that it is invalid to define boundaries for a linkage region by use of recombinants when the penetrance classes have two-way uncertainty, as ours do. The location of the maximum LOD score with D4S428 is  $\sim 8$  cM above the proximal boundary of the interstitial deletion and is, therefore, outside the marker chromosome (Grimbacher et al. 1999b). A primary reason for this discrepancy is a recombination in family 1, in the daughter who had a clinical score of 25 (fig. 1). This 4-year-old girl was given a diagnosis of HIES because of her newborn rash, moderate eczema, eosinophil count of  $940/\mu\text{l}$ , and serum-IgE level of 10,375 IU/ml (table 1). Although she may not be affected with HIES, despite these suggestive clinical and laboratory findings, it is also possible that she is affected, but that there is locus heterogeneity. In this case, the cause of HIES in family 1 would not be a mutation at the HIES locus on 4q. The discrepancy might also be explained by long-range effects on the expression of genes, located in the vicinity of D4S428, by control regions disrupted during the marker-chromosome formation.

Numerous genes have been mapped near D4S428, including c-kit and VEGFR-2, and  $>50$  genes are mapped to the 15–20-cM region involved in the interstitial deletion and marker-chromosome formation of the sporadic patient described above (Grimbacher et al. 1999b). However, on the basis of available functional information for these genes, there is no clear candidate gene for HIES.

Nonetheless, our new appreciation of the multisystem nature of the disease suggests that there are undiscovered links between the immune system and the connective-tissue and skeletal systems. Understanding the molecular pathology of HIES will shed light not only on inflammatory mechanisms, wound healing, and IgE regulation, but also on development of the face and midline structures, regulation of bone density, and aspects of connective tissue and dental exfoliation. Pursuit of disease gene(s) for HIES may lead to diagnostic and therapeutic approaches for individuals with this rare disease. Moreover, this information may also benefit patients with more-common medical problems related to the pathogenic mechanisms in HIES.

## Acknowledgments

The authors would like to thank Judi Miller and Roxanne Fischer for handling family data and samples, Amy P. Hsu for her help in establishing the genotyping procedure, and all of the families who participated in this study. B.G. was supported by the Deutsche Forschungsgemeinschaft grant GR1617/1-1 and an Immune Deficiency Foundation Research Fellowship Award (1998); H.D.O. was supported in part by the University of Washington Clinical Research Center (grant RR-37), NIH grant HD17427, the Immune Deficiency Foundation, and the Jeffrey Modell Foundation.

## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genemap'98, <http://www.ncbi.nlm.nih.gov/genemap98> (for markers and loci)  
 Online Mendelian Inheritance in Man, <http://www.ncbi.nlm.nih.gov/Omim> (for Job syndrome [MIM 147060] and hyper-IgE recurrent infection syndrome [MIM 243700])  
 Research Genetics Inc., <http://www.resgen.com> (for sex-averaged genetic map)  
 Whitehead Institute for Biomedical Research-MIT Center for Genome Research, <http://www-genome.wi.mit.edu> (for Whitehead-MIT genetic map)

## References

- Belohradsky BH, Daeumling S, Kiess W, Griscelli C (1987) Das Hyper-IgE-Syndrom (Buckley- oder Hiob-Syndrom). In: *Ergebnisse der Inneren Medizin und Kinderheilkunde*, Bd 55. Springer Verlag, Berlin pp 1–39
- Blum R, Geller G, Fish LA (1977) Recurrent severe staphylococcal infections, eczematoid rash, extreme elevations of IgE, eosinophilia, and divergent chemotactic responses in two generations. *J Pediatr* 90:607–609
- Borges WG, Hensley T, Carey JC, Petrak BA, Hill HR (1998) The face of Job. *J Pediatr* 133:303–305
- Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: individual and



- sex-specific variation in recombination. *Am J Hum Genet* 63:861–869
- Buckley RH (1996) Disorders of the IgE system: the hyper IgE syndrome. In: Stiehm ER (ed) *Immunologic disorders in infants and children*. 4th ed. Saunders, Philadelphia, pp 413–422
- Buckley RH, Becker WG (1978) Abnormalities in the regulation of human IgE synthesis. *Immunol Rev* 41:288–314
- Buckley RH, Sampson HA (1981) The hyperimmunoglobulinemia E syndrome. In: Franklin EC (ed) *Clinical immunology update*. Elsevier North-Holland, New York, pp 147–167
- Buckley RH, Sampson HA, Fisher PM, Becker WG, Shirley LR (1982) Abnormalities in the regulation of human IgE synthesis. *Ann Allergy* 49:67–72
- Buckley RH, Schiff SE, Hayward AR (1991) Reduced frequency of CD45RO<sup>+</sup> T lymphocytes in blood of Hyper IgE syndrome patients. *J Allergy Clin Immunol Suppl* 87:313 (abstract)
- Buckley RH, Wray BB, Belmaker EZ (1972) Extreme hyperimmunoglobulin E and undue susceptibility to infection. *Pediatrics* 49:59–70
- Claassen JJ, Levine AD, Schiff SE, Buckley RH (1991) Mononuclear cells from patients with the hyper-IgE syndrome produce little IgE when they are stimulated with recombinant human interleukin-4. *J Allergy Clin Immunol* 88:713–721
- Cohen MM (1988) Craniosynostosis update 1987. *Am J Med Genet Suppl* 4:99–148
- Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. *Am J Hum Genet* 53:252–263
- Davis SD, Schaller J, Wedgwood RJ (1966) Job's syndrome. Recurrent, "cold," staphylococcal abscesses. *Lancet* 1:1013–1015
- Donabedian H, Gallin JI (1983) The hyperimmunoglobulin E recurrent infection (Job's) syndrome. A review of the NIH experience and the literature. *Medicine* 62:195–208
- Dreskin SC, Gallin JI (1987) Evolution of the hyperimmunoglobulin E and recurrent infection (HIE, Job's) syndrome in a young girl. *J Allergy Clin Immunol* 80:746–751
- Dreskin SC, Goldsmith PK, Gallin JI (1985) Immunoglobulins in the hyperimmunoglobulin E and recurrent infection (Job's) syndrome. *J Clin Invest* 75:26–34
- Dreskin SC, Goldsmith PK, Strober W, Zech LA, Gallin JI (1987) Metabolism of Immunoglobulin E in patients with markedly elevated serum immunoglobulin E levels. *J Clin Invest* 79:1764–1772
- Farkas LG (1994) *Anthropometry of the head and face in medicine*. 2d ed. Raven Press, New York, pp 286
- Gahr M, Mueller W, Allgeier B, Speer CP (1987) A boy with recurrent infections, impaired PMN-chemotaxis, increased IgE concentrations and cranial synostosis: a variant of the hyper-IgE syndrome? *Helv Paediat Acta* 42:185–190
- Geha RS, Reinherz E, Leung D, McKee JT, Schlossmann S, Rosen FS (1981) Deficiency of suppressor T cells in the hyperimmunoglobulin E syndrome. *J Clin Invest* 68:783–791
- Grimbacher B, Holland SM, Puck JM (1998) The interleukin-4 receptor variant Q576R in hyper-IgE syndrome. *N Engl J Med* 338:1073–1074
- Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, Miller JA, et al (1999a) Hyper IgE syndrome with recurrent infections: an autosomal dominant multisystem disorder. *New Engl J Med* 340:692–702
- Grimbacher B, Dutra AS, Holland SM, Fischer RE, Pao M, Gallin JI, Puck JM (1999b) Analphoid marker chromosome in a patient with hyper-IgE syndrome, autism, and mild mental retardation. *Genetics in Medicine* (in press)
- Hershey GKK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA (1997) The association of atopy with a gain-of-function mutation in the  $\alpha$  subunit of the interleukin-4 receptor. *N Engl J Med* 337:1720–1725
- Hill HR, Ochs HD, Quie PG, Clark RA, Pabst HF, Klebanoff SJ, Wedgwood RJ (1974b) Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. *Lancet* 2:617–619
- Hill HR, Quie PG (1974a) Raised serum IgE levels and defective neutrophil chemotaxis in three children with eczema and recurrent bacterial infections. *Lancet* 1:183–187
- Holland SM, Gallin JI (1998) Disorders of granulocytes and monocytes. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, et al. (eds) *Harrison's principles of internal medicine*. 14th ed. McGraw-Hill, New York, pp 351–356
- King CL, Gallin JI, Malech HL, Abramson SL, Nutman TB (1989) Regulation of immunoglobulin production in hyperimmunoglobulin E recurrent-infection syndrome by interferon- $\gamma$ . *Proc Natl Acad Sci USA* 86:10085–10089
- Kirchner SG, Sivit CJ, Wright PF (1985) Hyperimmunoglobulinemia E syndrome: Association with osteoporosis and recurrent fractures. *Radiology* 156:362
- Kraemer MJ, Ochs HD, Furukawa CT, Wedgwood RJ (1982) In vitro studies of the hyper-IgE disorders: suppression of spontaneous IgE synthesis by allogenic suppressor T lymphocytes. *Clin Immunol Immunopathol* 25:157–164
- Kraemer MJ, Ochs HD, Klebanoff SJ, Altman LC, Wedgwood RJ (1981) The inheritance of Job syndrome. *Clin Res Suppl* 29:123A
- Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus analysis in humans. *Proc Natl Acad Sci USA* 81:3443–3446
- Ochs HD, Kraemer MJ, Lindgren CG, Furukawa CT, Wedgwood RJ (1983) Immune regulation in the hyper-IgE/Job syndrome. In: *Birth defects: original article series* (March of Dimes Birth Defects Foundation) 19:57–61
- Ott J (1989) Simulation methods in human linkage analysis. *Proc Natl Acad Sci USA* 86:4175–4178
- Ott J (1991) *Analysis of human genetic linkage*. Johns Hopkins University Press, Baltimore, pp, 49–51, 191
- Paganelli R, Scala E, Capobianchi MR, Fanale-Belasio E, D'Offizi G, Fiorilli M, Aiuti F (1991) Selective deficiency of interferon-gamma production in the hyper-IgE syndrome. Relationship to in vitro synthesis. *Clin Exp Immunol* 84:28–33
- Rousset F, Robert J, Andary M, Bonnin JP, Souillet G, Chretien I, Briere F, et al (1991) Shifts in interleukin-4 and interferon- $\gamma$  production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J Allergy Clin Immunol* 87:58–69

- Schäffer AA, Gupta SK, Shriram K, Cottingham RW Jr (1994) Avoiding recomputation in linkage analysis. *Hum Hered* 44: 225–237
- Stewart EA, McKusick KB, Aggarwal A, Bajorek E, Brady S, Chu A, Fang N, et al (1997) An STS-based radiation hybrid map of the human genome. *Genome Res* 7:422–433
- Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. *Am J Hum Genet* 56: 777–787
- Terwilliger JD, Ott J (1994) *Handbook of human genetic linkage*. Johns Hopkins University Press, Baltimore, pp 77–78
- Van Epps DE, El-Naggar A, Ochs HD (1983) Abnormalities of lymphocyte locomotion in immunodeficiency disease. *Clin Exp Immunol* 53:678–688
- Van Scoy RE, Hill HR, Ritts RE, Quie PG (1975) Familial neutrophil chemotaxis defect, recurrent bacterial infections, mucocutaneous candidiasis, and hyperimmunoglobulinemia E. *Ann Intern Med* 82:766–771
- Vercelli D, Jabara HH, Cunningham-Rundles C, Abrams JS, Lewis DB, Meyer J, Schneider LC, et al (1990) Regulation of Immunoglobulin (Ig) E synthesis in the hyper-IgE syndrome. *J Clin Invest* 85:1666–1671
- Weeks DE, Ott J, Lathrop GM (1990) SLINK: a general simulation program for linkage analysis. *Am J Hum Genet Suppl* 47:A204